Seasonal Prevalence and Transmission of Salivary Gland Hypertrophy Virus of House Flies (Diptera: Muscidae)

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ABSTRACT A survey (2005–2006) of house fly, *Musca domestica* L. (Diptera: Muscidae) populations on four Florida dairy farms demonstrated the presence of flies with acute symptoms of infection with salivary gland hypertrophy (SGH) virus on all farms. Disease incidence varied among farms (farm averages, 0.5–10.1%) throughout the year, and it showed a strong positive correlation with fly density. Infections were most common among flies that were collected in a feed barn on one of the farms, especially among flies feeding on wet brewers grains (maximum 34% SGH). No infections were observed among adult flies reared from larvae collected on the farms, nor among adults reared from larvae that had fed on macerated salivary glands from infected flies. Infected female flies produced either no or small numbers of progeny, none of which displayed SGH when they emerged as adults. Healthy flies became infected after they fed on solid food (a mixture of powdered milk, egg, and sugar) that had been contaminated by infected flies (42%) or after they were held in cages that had previously housed infected flies (38.6%). Healthy flies also became infected after they fed on samples of brewers grains (6.8%) or calf feed (2%) that were collected from areas of high fly visitation on the farms. Infection rates of field-collected flies increased from 6 to 40% when they fed exclusively on air-dried cloth strips soaked in a suspension of powdered egg and whole milk. Rates of virus deposition by infected flies on food were estimated by quantitative polymerase chain reaction at ≈100 million virus copies per fly per hour. Electron microscopy revealed the presence on enveloped virus particles in the lumen of salivary glands and on the external mouthparts of infected flies.

KEY WORDS house fly, *Musca domestica*, salivary gland hypertrophy virus, infection

Salivary gland hypertrophy (SGH) virus (SGHV) of house flies, *Musca domestica* L. (Diptera: Muscidae) *Md*SGHV is a nonoccluded, enveloped, rod-shaped double-stranded DNA virus that was first discovered in fly populations in Florida (Coler et al. 1993). The virus seems to be most closely related to SGHVs that infect the fly *Merodon equestrus* (F.) and *Glossina* spp. (Jaenson 1978, Amargier et al. 1979). Infected flies of all fly species display grossly enlarged salivary glands in both sexes, and virus particles are thought to be deposited when infected flies feed. Healthy flies presumably acquire the infection when they feed on contaminated food substrates.

Recently, we characterized the effects of *Md*SGHV infection on reproductive fitness of male and female house flies (Lietze et al. 2007). Females that are infected as young (previtellogenic) flies do not mate or develop eggs. Protein digestion occurs as in healthy flies, but infection blocks production of female-specific proteins involved in egg maturation. Females that are infected later in life (after developing eggs) deposit their current batch of eggs, but they do not undergo additional oogenic cycles. Infected male flies are less avid and less successful in attempts to mate and transfer sperm than control males. The *Md*SGHV is not transferred from infected males to healthy females during mating, and infected flies of both sexes do not live as long as healthy flies (Lietze et al. 2007).

Very little is known about *Md*SGHV transmission patterns or epizootiology. Seasonal fluctuations of infection in wild house fly populations were observed in 1991 at a single Florida dairy; in these collections, the incidence of salivary gland virus (SGHV) varied from 1.5 to 18.5% between May and October with peaks produced in June, August, and October (Coler et al. 1993). Initial attempts at per os transmission of *Md*SGHV resulted in fairly low infection rates, whereas injection of *Md*SGHV into the thorax results in 100% of the flies displaying SGH within 4–6 d (Coler et al. 1993, Lietze et al. 2007).

The objectives of the current study were to 1) observe seasonal prevalence of *Md*SGHV infections on several farms over two fly seasons; 2) determine whether infection prevalence is related to fly densities; 3) determine whether the virus is transmitted vertically; 4) evaluate oral (horizontal) transmission under a range of food substrate conditions; 5) determine rates of virus particle deposition by infected flies; and 6) to document, by electron microscopy, the pres-

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ence of virus particles in the lumen of salivary glands and on the mouthparts of infected flies.

Materials and Methods

Prevalence of *Md*SGHV in Field Populations. Adult house flies were collected from Florida dairy farms in 2005–2006. In 2005, flies were collected weekly from May through December from three farms in Alachua and Gilchrist counties (Florida). Collections in 2006 included an additional farm (farm 4), and they were made from March through November. Farm 1 was a large commercial dairy operation with ≈6,000 milking animals. Flies on this farm were collected from the feed storage barn, especially around large piles of wet brewers grains, which were delivered several times daily from a nearby brewery. Farm 2 was a smaller farm with ≈600 milking animals; flies were collected around feeders and in the feed storage barns. Farm 3 was a dairy operated by the University of Florida with ≈1,000 milking animals; flies were collected in the calf barns that housed 30–60 calves depending on the time of year. Farm 4 (2006 collections only) was a small family farm with ≈400 milking animals.

Flies were collected with sweep nets and placed immediately in cages provided with water and food (a 6:6:1 mixture [by volume] of powdered milk, sucrose, and dried egg). Cages were returned to the laboratory and held at 20°C for up to 4 d before being examined for infection. These holding conditions prevented any possible new infections arising from horizontal transmission in the cages from contributing to survey results (C.J.G., unpublished observations). Flies were dissected in a 0.6% saline solution and examined for the enlarged salivary glands that characterize acute infection with this virus (Coler et al. 1993). One hundred flies from each farm per week were examined for infection except in some of the early and late season collections when fly densities were low. Differences among farms were compared by one-way analysis of variance (ANOVA) by using the GLM procedure of the Statistical Analysis System (SAS) (SAS Institute 1992). In all of the analyses, percentage of infection data were subjected to arcsine transformation before analysis (Kutner et al. 2005). Means (by farm) were separated using the Means/Tukey statement of SAS. Possible differences between male and female infection rates were examined separately for each farm by one-way ANOVA.

Results of the 2005 survey indicated that SGHV infection was substantially more common in flies collected from farm 1, where flies were collected near wet brewers grains. This was the only farm that used this ingredient in its feeding program. In October–November 2006, flies were collected from an additional site on farm 1 to determine whether there were local differences in infection rates. On these dates, flies were collected from the brewers grains as usual and also from the calf barns located ≈1 km from the grain storage barn. Infection rates from the two sites were evaluated by two-way ANOVA by using collection site, sex, and site × sex as the grouping variables with PROC GLM of SAS. In addition, possible fly sex ratio differences between the two collection sites were compared by one-way ANOVA.

Fly Abundance and SGHV. Observations during 2005 suggested that SGHV prevalence was correlated with fly density. To document this, instantaneous estimates of relative fly abundance at the time of collection were made in 2006 by using Scudder grid counts (Scudder 1947). Grid counts were made at 10–12 sites per farm on each visit by photographing the grids with a digital camera 5 s after placing the grids in areas where flies were aggregated. The relationship between fly density and SGHV infection was evaluated by regression using PROC REG of SAS.

Possible Acquisition of SGHV by Fly Immatures. The possibility that adult flies acquire SGHV as immatures was evaluated using three approaches. In the first approach, natural infection of immatures was assessed by making collections of mature fly larvae from three farms during 3 wk in July 2005 when infection levels in adults were relatively high. Larvae were held in the laboratory, allowed to pupate and emerge, given food and water, and adults were examined for infection 7 d after emergence. In the second approach, three groups of 25 laboratory-reared second instars were placed on 5 cm^3 of larval rearing medium (Hogsette 1992) to which had been added 12 salivary gland pairs dissected from infected flies that had been homogenized in 0.5 ml of 0.6% saline. Glands for these assays were removed from field-collected flies on the day of the test. An additional set of larvae was treated with homogenized glands from uninfected flies as controls. The experiment was repeated on three separate occasions with different sets of glands. Larvae were held for adult emergence, and flies were assessed for infection 1 wk after emergence. In the third approach, groups of 25 adult female flies were infected with SGHV on either day 1 or day 3 after emergence by injection by using the methods described by Lietze et al. (2007) and placed in cages with 25 uninfected male flies. An additional set of 25 females injected with only saline was used as healthy controls. Flies were held at 25°C with food and water, and they were provided with spent fly larval rearing medium as an oviposition substrate on days 6, 8, and 11 after emergence. Oviposition media were removed from the cages after 24 h and placed on fresh larval medium. Larvae were held for fly emergence, and adults were dissected 7 d after emergence to determine whether F1 progeny of virus-injected flies were infected. Dissection of parental flies on day 11 confirmed that all of the virus-injected flies were infected with SGHV.

Oral Transmission: Contaminated Laboratory Food and Cages. Initial testing of oral transmission among adult flies was conducted with field-collected flies from farm 1. Flies (≈2,000) from farm 1 were collected on 11 July and 2 and 9 September 2005, and they were held for 1 wk at 25°C in cages provided with water-soaked cotton balls and food (sucrose/milk/egg as described above). The incidence of SGHV infection in these three fly collections was calculated to be 23, 19, and 18%, respectively. The flies, contaminated food,

and contaminated water were removed from the cages after 7 d. Groups of 400 newly emerged, uninfected flies were then placed in cages with either contaminated food and water dishes, contaminated food only (plus clean water), contaminated water only (plus clean food), or in contaminated cages with clean water and food (one cage per treatment, tested on three separate occasions with different fly collections from the field). Controls consisted of flies placed in cages with food and water that had been exposed to healthy flies for 7 d. Flies were dissected and examined (100 flies per treatment) for infection 7 d after being placed in test cages.

In a subsequent experiment, groups of 38–243 newly emerged flies were injected with SGHV, and they were held in cages with food and water for 7 d, then removed. Groups of 100–150 healthy flies were placed either in clean cages with contaminated food (and clean water) or with clean food and water in contaminated cages. In addition, a group of healthy flies was fed ad libitum on 10% sucrose containing one infected gland pair (homogenized) per milliliter. Control flies were either held with clean food, water, and cages or fed on sucrose containing no virus. Flies were assessed for infection 10 d after exposure to virus treatments. The experiment was repeated three to five times.

Oral Transmission with Different Food Substrates. Samples of fly food sources were collected from farms 1 and 3 (brewers grains and calf feed pellets, respectively) on three occasions in May–June 2006. The samples, 10 cm^3 each, were taken from areas that were being actively visited by flies at the time of collection. Each sample was placed individually in cages with 50 1-d-old laboratory-reared flies per cage and water. After 24 h, normal colony fly food was added to the cages, and flies were held for 7 d at 25°C before being dissected and examined for SGHV infection. As controls, laboratory flies were allowed to feed on brewers grains (farm 1) or calf feed (farm 3) that had been protected from fly feeding. In total, 26 and 13 food samples were collected on three occasions from farms 1 and 3, respectively.

In the next test, 10-cm^3 samples of three types of food sources that had been protected from fly feeding (brewers grains, calf feed pellets, and calf manure) were collected from farm 1 on 6 July 2006. Four samples of each food type were placed in cages of 2,000 flies that were collected from the farm on day of food sample collections. An additional cage of field flies was presented with four dishes of granulated sucrose (table sugar). Finally, a cage also was presented with four, 2–6-cm strips of cotton muslin that had been dipped in a slurry of water, powdered egg, and powdered whole milk (3:1:1) and allowed to dry. The protein strips were suspended from the top of the cage by paper clips. The flies were confined with the test food substrate and water for 24 h, after which the food items were removed and transferred to clean cages with 50 young laboratory flies that had been starved for 24 h previously (one food item per cage/four cages per food type). Flies were held for 7 d, and then they were dissected and examined for SGHV infection.

An experiment was conducted to determine whether infection rates of field-collected flies could be amplified by holding them with a food substrate that would promote cofeeding by infected and uninfected flies in proximity. Cages of 2,000 flies were collected from farm 3 on three occasions in July and August 2006, and they were provided with water and strips of muslin that been dipped in egg/milk slurry and dried as the only food source. As described above, the strips were suspended from the roof of the cage. New strips were added to the cages daily for 7 d. Samples of 100 flies were removed, dissected, and assessed for SGHV infection on the day of collection and on days 3 and 7 after collection.

Quantitative Polymerase Chain Reaction (qPCR) of SGHV Released on the Feeding Substrate. Viremic flies were produced by injection of filter-sterilized infected gland homogenates into 1-d old flies as described previously (Lietze et al. 2007). Injected test flies and saline-injected control flies (25 flies per cage) were maintained in separate cages under constant conditions for 6 d to allow full expression of SGHV symptoms. Two control and four test cages were set up.

Before the experiment, healthy control flies and viremic test flies were deprived of food but not water for 24 h. A filter strip (1 by 3 cm), previously soaked in a 20% sucrose solution and air-dried for 10 min, was placed in each of the cages, and it was exposed to the flies for 30 min. The filter strips were then removed, cut in halves, and processed for PCR detection of viral DNA. As a positive control, one half of each control strip was "spiked" with 25 μl of a viremic gland preparation (with a concentration of 10 IgE/ml). Each strip was placed in a microcentrifuge tube with 500 μl of TE buffer and mixed for 30 s. A second control included 25 μl of the viremic gland preparation mixed in 500 μl of TE buffer. Strips were removed and all samples were boiled for 5 min. These stock samples were diluted 10-fold and 100-fold. One microliter of each stock and dilution were used per 25-μl PCR reaction.

A series of qPCR primers were designed from selected *Md*SGHV sequences (C10, a putative thymidylate synthetase, E valve = 0; and the F05, unknown) by using primer3 freeware to be 20–22 nucleotides, contain 50% GC, and possess a 60°C T_m to produce single bands ranging from 100 to 150 bp. Three of the four primer pairs tested (qC10 F+R ATTTCGGT GCTCGGTACATC and CGTCGACTACTCGGCT CATATT; qF05 F+R CGGCATACGACAGAAACT CATC and AGAACTGGGTTGCTATCGCTTC; and qC10a+R FAGAGTTTGGGCCCCATTTAC and GTCGACTACTCGGCTCATATTG) produced a single product from the DNA samples extracted from the filter paper sample. Sequencing the amplified products confirmed that the products were identical to the target sequences in the template C10 and F05 sequences. Quantitative PCR, by using three different primer sets, was conducted initially on a sample of purified virus DNA. Relative copy number present in

these samples was estimated by measuring total DNA with UV absorbance (SmartSpec Plus, Bio-Rad, Hercules, CA) and by using an estimated mass of the *Md*SGHV (≈0.15 fg per copy). Serial 10-fold dilutions of viral DNA were amplified using the iCycler iQ real-time PCR detection system with iQ SYBR Green Supermix (Bio-Rad). The stock preparations obtained from a filter strip previously exposed to 25 viremic female house flies was included in the qPCR template. All samples were analyzed using the iCycler iQ real-time PCR detection system with iQ SYBR Green Supermix.

Electron Microscopy. House flies were injected with viral inoculum 1 d after emergence and held with food and water for 6 d as described above. Infected and control females were dissected in fixative and salivary glands were fixed for 2.5 h at room temperature in 2.5% glutaraldehyde and 2% paraformaldehyde (in 0.1 M cacodylate buffer), washed three times in 0.1 M cacodylate buffer (15 min each), and postfixed for 1 h in 1% osmium tetroxide. After three washes in sterilized water (10 min each), the fixed organs were dehydrated in a graded ethanol series (25, 50, 75, 100, 100% ethanol; 10 min each). Alcohol was exchanged with 100% acetone, and tissues were embedded in Epon-Araldite.

Thin sections were mounted on Formvar-coated nickel grids. Sections were poststained with uranyl acetate followed by lead citrate and examined with a Hitachi H-7000 electron microscope (Hitachi, Tokyo, Japan).

Additional infected and healthy flies were removed from their respective cages and immediately immersed into 2.5% glutaraldehyde and 2% paraformaldehyde (in 0.1 M cacodylate buffer) for 24 h at 4°C. Flies were dehydrated in an ethanol series and critical-point dried by using a Bal-Tec 030 critical point dryer (Bal-Tec, Witten, Germany). Dried specimens were mounted onto the stubs and dissected to expose labial palps. Specimens were sputter coated with Au/Pt alloy and examined on a Hitachi 4000 FE-SEM operating at 4–6 kV. Measurements of all digitally captured subjects were made using SPOT software 3.4.3 (Diagnostic Instruments, Sterling Heights, MI).

Results

Prevalence in Field Populations. *Md*SGHV infection levels in 2005 were highest in July, with a maximum infection of 34% observed on 3 July on farm 3 (Fig. 1). Infection rates were generally low (<10%) during the rest of 2005 except on farm 1. At least some infected flies were collected through 20 November, but no infections were observed after that date. In 2006, farm 1 was the only farm where infections were found consistently throughout the fly season, and infection rates were >5% from most of the collections on this farm. Infection rates were <3% in almost all of the other collections from the remaining farms. Overall, ≈10% of flies collected from both years on farm 1 were infected; infection rates on this farm were significantly higher in male (13.3%) than in female flies (6.7%) (Table 1). In contrast, infection rates between male and female flies did not differ on the other farms and averaged 3.1, 1.2, and 0.5% overall on farms 2, 3, and 4, respectively (Table 1).

Flies collected near the piles of wet brewers grains on farm 1 had significantly higher *Md*SGHV infection rates (12.1 and 3.5% for males and females, respectively) than flies that were collected from the calf barn on the same farm (2.0 and 0.4% for males and females, respectively) (Table 2). Infection rates were also significantly higher among male than female flies at both locations. Flies collected from the brewers grains had a significantly higher percentage of female flies (64.8%) than flies collected from the calf barn (51.4%).

Fly Abundance and *Md*SGHV. There was a significant correlation between relative fly abundance and SGHV infection in the 2006 collections (Fig. 2). Fly abundance explained 22% of the variation in infection rates ($F = 29.16$; df = 1, 156; $r^2 = 0.2206$). Parameter estimates for the regression model were 0.46 ± 0.76 for the intercept and 0.133 ± 0.02 for the slope.

Possible Acquisition of SGHV by Fly Immatures. In total, 3,200 adult flies emerged from larvae that were collected from the farms in July 2005; none of the 300 flies that were examined showed symptoms of SGHV infection (not presented in a table). None of the flies that emerged from larvae that fed on homogenized salivary glands from infected flies were infected (not presented in a table). Adult flies that were injected with virus within 24 h of emergence did not produce any progeny during the experiment. Flies that were infected on day 3 after emergence produced an average of 15.8 F1 progeny per female on day 6, none of which expressed overt symptoms of with SGHV, and they did not produce any additional progeny on days 8 and 11 (data not presented in a table). Uninfected injected controls produced 86.2, 74.6, and 61.4 F1 adults on days 6, 8, and 11, respectively (total, 222 progeny per female). None of the progeny of the control flies displayed SGH symptoms.

Oral Transmission: Contaminated Laboratory Food and Cages. Laboratory flies that were held with food or food and water that had been visited by field-collected flies displayed significantly higher SGHV infection rates (9.9 and 8.0%, respectively) than flies that were held with contaminated water from cages of field-collected flies (4.9%) or flies that were held in contaminated cages with clean food and water (3.7%) (Table 3). None of the control flies became infected. Laboratory flies that were held with either food or in the cages that had housed flies that were injected with virus in the laboratory became infected to a similar degree (42 and 38.6%, respectively; $P > 0.05$), and their infection rates were not significantly different from flies that had fed on 10% sucrose containing the equivalent of one infected gland pair per milliliter (50%) (Table 4).

Oral Transmission with Different Food Substrates. Of the 26 samples of brewers grains collected from high fly activity sites on farm 1, 18 (69%) produced infections of at least one healthy colony fly that fed on

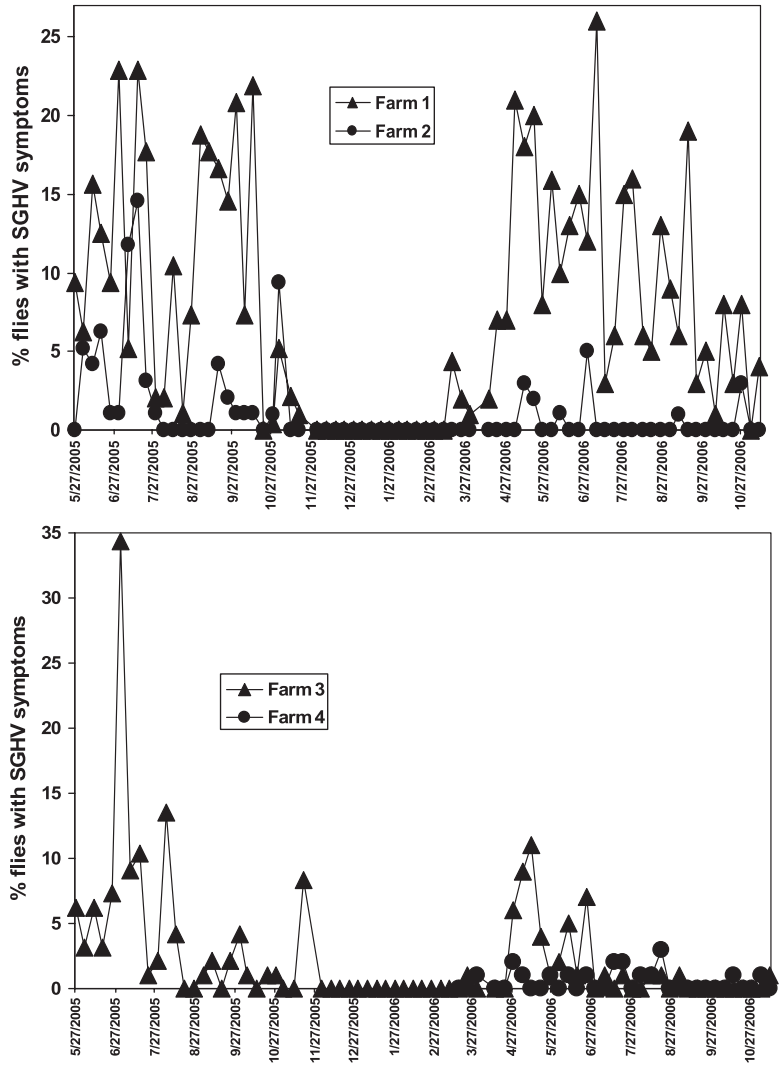

Fig. 1. Seasonal prevalence of adult house flies expressing symptoms of *Md*SGHV on Florida dairy farms in 2005 and 2006.

the samples in the laboratory, for an overall infection rate of 6.8% (Table 5). Of the samples of calf feed pellets collected from farm 3, five of the 13 collections (38%) produced infections of at least one colony fly in the laboratory, for an overall infection rate of 2.0%. None of the flies that fed on uncontaminated samples of brewers grains of calf feed pellets became infected.

Attempts to inoculate dry food substrates by exposing them to field-collected flies for 24 h resulted in generally low rates of transmission to laboratory flies (Table 6). None of the flies that fed on treated brewers grains became infected, and infection rates recorded when using calf manure, calf feed, and dry sucrose were 0.8, 1.8,, and 4.2%, respectively, with no significant differences among these four food substrates. The most effective food substrate was cotton strips that had been dipped in egg/milk slurries and allowed to dry; this substrate resulted in significantly higher in-

Table 1. Overall prevalence of SGHV symptoms in adult house flies from four Florida dairy farms in 2005–2006

	Mean (SE) % SGHV infection status of flies collected from				ANOVA
	Farm 1	Farm 2	Farm 3	Farm 4	*F* (among farms)
Infected ♀♀	6.7 (0.7)a	1.2 (0.2)b	2.9 (0.7)b	0.6 (0.2)b	19.13**[a]
Infected ♂♂	13.3 (1.4)a	1.3 (0.4)b	3.2 (1.0)b	0.4 (0.2)b	38.81**
ANOVA *F* (♂♂ vs. ♀♀)	17.44**[b]	0.26 ns	0.89 ns	0.85 ns	

[a] Means within rows followed by the same letter are not significantly different at $P < 0.05$; **, $P < 0.01$; df = 3, 195.
[b] ns, $P > 0.05$; **, $P < 0.01$; df = 1, 119 for farms 1, 3, and 4; df = 1, 38 for farm 2.

Table 2. Prevalence of SGHV symptoms and sex composition of adult house flies collected from wet brewers grains and from calf barn of farm 1 during October–November 2006

	Mean (SE) % SGHV infection status and % female flies	
	Wet brewers grains	Calf barn
Infected ♀♀	3.5 (1.7)	0.4 (1.0)
Infected ♂♂	12.1 (8.4)	2.0 (1.5)
% Female flies	64.8 (3.1)	51.4 (1.9)
	ANOVA *F*	
SGHV infection[a]		
♂♂ vs. ♀♀ inf	12.11**	
Collection site	25.78**	
Sex × collection site	0.72 ns	
% Females (collection site)[b]	14.01**	

[a] $P < 0.01$; ns, $P > 0.05$; numerator df = 1, 1,1; error df = 16.
[b] **, $P < 0.01$; df = 1, 8.

fection rates (10%) than any of the others tested (Table 6). When flies were collected from farm 1 and held with dried egg/milk strips as the only food source, there was no significant difference in infection rates between day 1 (5.9% for males, 6.5% for females) and day 3 (10.5% for males, 7.7% for females) after collection (Table 7). However, by day 7, SGHV infection rates had increased significantly for both males (39.7%) and female flies (40.9%).

qPCR of SGHV Released on the Feeding Substrate. The qPCR reactions were conducted in triplicate for the three different primer sets. Plotting the log of copy numbers against mean threshold cycle numbers produced similar standard curves with all three primer sets (Table 8). The stock preparation obtained from a filter strip previously exposed to 25 viremic female house flies was included in the qPCR template. Using the generated standard curves, qPCR reactions estimated that infected house fly females deposited $\approx 9.8 \pm 1.47 \times 10^7$ virus particles per hour (Table 8).

Electron Microscopy. In the hypertrophic glands, the rod-shaped nucleocapsids measuring 550- by 20-nm exit the nuclei (Fig. 3A), assemble their outer envelope in the cytoplasm (≈650 by 75 nm; Fig. 3A and B), and migrate to the luminal surface, where they bud off into the salivary gland lumen as enveloped nonoccluded virus particles (Fig. 3D and E). Relatively few particles are observed in the cytosol adjacent to the hemocoel face (Fig. 3C), suggesting a movement is being directed by host cues. The massive numbers of SGHV that accumulate in the lumen are presumed to be released during feeding. Scanning electron microscopy examination of the cuticular surface of the labellum reveal rod-shaped particles measuring ≈550 by 70 nm (Fig. 4). These particles, thought to be released SGHV particles, support our contention that this virus is horizontally transmitted by surface contamination of shared food substrates.

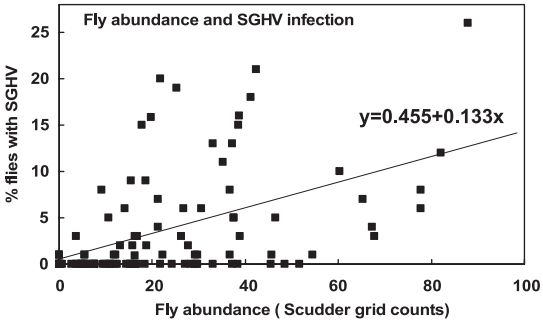

Fig. 2. Relationship between fly relative abundance and prevalence of *Md*SGHV symptoms in house flies on Florida dairy farms in 2006.

Table 3. SGHV infection of adult house flies 7 d after feeding exposure to food, water, and cages that had housed ≈2,000 flies collected from farm 1 for 7 d

Virus treatment	Mean (SE) % flies with SGHV[a]
Contaminated food and water	9.9 (0.5)a
Contaminated food only	8.0 (0.7)a
Contaminated water only	4.9 (0.9)b
Contaminated cage only	3.7 (0.8)b
Clean cage/food/water controls	0.0 (0.0)c

[a] Means followed by the same letter are not significantly at $P = 0.05$ (Tukey's method). ANOVA: $F = 70.65$; df = 4, 10; $P < 0.01$).

Discussion

The incidence of SGHV varied widely among farms and at different times of the year. A substantial amount of this variation could be attributed to fluctuations in fly density (Fig. 2). This observation is consistent with the hypothesis that transmission is primarily horizontal among cofeeding adult flies. The quantity and quality of food resources available to flies in the form of animal feeds and manure are somewhat stable on a given dairy farm throughout the year. Flies are highly aggregated on preferred food sources, with groups of flies congregating around "hot spots" within such preferred items (Fig. 5). High fly populations therefore increase the probability of infected and uninfected flies feeding in proximity at the same time.

Fly populations collected from the feed barn of Farm 1 had consistently higher rates of SGHV than flies collected from the other farms and from a different site (calf barn) on the same farm (Table 2). The most conspicuously unique feature of this collection site is the large deposits of wet brewers grains that are

Table 4. SGHV infection of adult house flies 10 d after feeding on 10% sucrose containing virus or after exposure to food and cages that had housed 38–243 laboratory-infected flies for 7 d

Virus treatment	No. reps	No. flies examined	Mean (SE) % flies with SGHV
Oral (1 IgE/ml)	3	296	50.0 (9.0)
Contaminated cage	5	410	38.6 (7.8)
Contaminated food	4	310	42.0 (8.9)
Oral controls	3	300	0.0
Clean cage/food controls	3	300	0.0

ANOVA: ***F*** among treatments with virus = 0.42 (df = 2, 9; $P > 0.05$).

Table 5. SGHV infections of laboratory-reared house flies 7 d after feeding on food samples (brewers grains and calf feed) collected from areas of high fly activity on farms 1 and 3 during June 2006

Farm and food type	No. food samples collected	No. samples positive for SGHV	Mean (SE) % flies infected after feeding on samples
Farm 1, brewers grains	26	18	6.8 (1.3)
Farm 3, calf feed	13	5	2.0 (0.9)

delivered several times per day (Fig. 5). We collected flies from around the grains because that is where fly populations were most heavily concentrated when we typically visited the farm (early afternoon). However, house fly distribution and abundance patterns are known to vary throughout the day (Murvosh and Thaggard 1966, Raybould 1966). Casual observations made at other times of day indicated that fly distribution patterns at this site change depending on the time of day and weather conditions. For example, flies were often seen resting (but not feeding) on baled hay in the feed barn during early morning and late afternoon. At other times, especially after rain, flies were concentrated on moist margins of other feed ingredients such as corn and soy meal. Some of these microhabitats may be more conducive to virus deposition, survival, and uptake than others.

Our results with collections of fly larvae from the field, attempted virus transmission to larvae, and attempted transmission of virus from infected females to their progeny all argue strongly against vertical transmission in this disease system. This is not surprising because a major feature of SGHV infection is a shutdown of ovarian development (Lietze et al. 2007). In the vertical transmission experiment presented here, we attempted to maximize the possibility of viral transfer to progeny by infecting females part way through ovarian development so that they would produce at least some offspring, but none of the small number of progeny from infected mothers were infected. These results also were observed in long-term studies of the effect of infection on fly reproductive fitness (Lietze et al. 2007). Vertical transmission therefore is highly unlikely to play a role in the epizootiology of SGHV.

Therefore, horizontal transmission among adult flies seems to be the primary infection route. Cage studies demonstrated that the virus could be transmitted by allowing healthy flies to feed on contaminated food (Tables 3 and 4). Further confirmation of the importance of this mechanism came when we were able to infect healthy flies by allowing them to feed on contaminated food collected from the field (Table 5). Because house flies must regurgitate on solid food before they can feed on it, we expected that this would be an important component of SGHV transmission patterns. More surprising was the observation that healthy flies became infected when held with clean food and water in cages that had previously held infected flies. Infection in these cases may have been by incidental ingestion of virus by flies during grooming behavior. However, fly feces also are known to be attractive to several muscoid species, including house flies, and some flies will feed actively on feces to supplement dietary protein needs (Mayer et al. 1972, Stoffolano et al. 1995, Carlson et al. 2000). Flies rest in aggregated and predictable patterns in the field to a degree that fecal spot accumulations can be used to estimate fly population sizes (Lysyk and Axtell 1985). Moreover, flies with undeveloped ovaries deposit approximately four-fold as many fecal spots as flies with more-developed ovaries (Sasaki et al. 2000). Our results suggest that fly feces and regurgitation deposits could provide an additional vehicle for SGHV transmission in the field. Further work is needed to document whether the virus remains viable after passage through the gut, and, if so, to determine virus survival times after being deposited by host flies.

Tests using different contaminated food substrates resulted in fairly low transmission rates, and no infections were observed with contaminated brewers grains (Table 6). The reasons for these low rates are not clear. It may be that the numbers of infected virus donor flies was too low, the exposure time of foods to donor flies too short, or that the volume of food provided to the flies was too great. Strips of cotton with air-dried egg and milk slurry gave the highest transmission rates, and these strips were able to amplify

Table 6. SGHV infection of laboratory-reared house flies 7 d after a 24-h exposure to different foods that had been fed on for 24 h by flies collected from farm 1 in July and August 2006

Food source	Mean (SE) flies with SGHV
Calf manure	0.8 (0.8)a
Brewers grains	0.0 (0.0)a
Calf feed	4.2 (1.1)a
Sucrose	1.8 (0.6)a
Protein strip[a]	10.0 (4.1)b
ANOVA F^b	29.42**

[a] Strips (2 by 6 cm) of cotton muslin soaked in egg/milk slurry and allowed to dry.

[b] df = 4, 15; **, $P < 0.01$; means followed by the same letter are not different at $P = 0.05$ (Tukey's method).

Table 7. SGHV infection of field collected house flies at 1, 3, and 7 d after being held with water and proteinaceous (milk/egg) food strips

Days after collection	Mean (SE) % flies infected with SGHV		
	Males	Females	Overall
Day 1	5.9 (1.0)a	6.5 (2.5)a	6.0 (1.7)a
Day 3	10.5 (2.3)a	7.7 (1.5)a	8.7 (1.3)a
Day 7	39.7 (5.6)b	40.9 (5.2)b	40.3 (5.4)b
ANOVA F^a	31.6**	28.6**	34.4**

[a] df = 2, 6; means within columns followed by the same letter are not different at $P = 0.05$ (Tukey's method).

Table 8. Calculation of total *Md*SGHV copy numbers on a filter paper strip exposed to 25 viremic females for 30 min

	Primer			
	qC10F+R	qF05F+R	qC10aF+R	Mean (SD)
F(x)	−3.39× + 34.95	−3.17× + 33.20	−3.36× + 34.14	n/a
y	17.55	16.23	16.60	n/a
x	5.13	5.36	5.22	n/a
6.8431 × e^x (copies/μl)	9.24e5	1.55e6	1.14e6	1.21e6 (0.32e6)
Copies/fly/h	7.3e07	1.24e8	9.14e7	9.68e7 (1.47e7)

n/a, not applicable.

infection rates in collections of field-collected flies (Tables 6 and 7). Virus survival times outside the host may be affected by the quality of the food substrate on which it is deposited, but this has not been investigated.

It has been presumed that the primary means of transmission of house fly SGHV is by deposition of virus from salivary secretions during feeding. Results with qPCR demonstrated that actively feeding flies deposit an estimated 100 million virus copies per hour on the food substrate. Moreover, enveloped virus particles were visualized by electron microscopy in the lumen of the glands and on the external mouthparts of infected flies. Infection of the narcissus bulb fly may occur by a similar mechanism, because these flies have comparable feeding mechanisms and forage on nectar and pollen (Conn 1976, Gilbert 1985, Finch et al. 1990). Horizontal transmission mechanisms in *Glossina* spp. remains uncertain. It has been reported that this virus is transmitted maternally via infected milk gland to progeny larvae (Jura et al. 1989). Tsetse fly adults feed exclusively on blood but are attracted preferentially to certain kinds of plants when they seek shelter during hot times of the day (Syed and Guerin 2004). Resting sites could provide an opportunity for horizontal transmission in addition to cofeeding of infected and uninfected flies on mammalian hosts. It should be noted that in tsetse fly mass-rearing programs, adults are fed en masse on membranes, thereby enhancing the potential for horizontal transmission (Abd-Alla et al. 2007).

In summary, SGHV infection occurs throughout the year in Florida house fly populations on dairy farms, and it is spread primarily, if not exclusively, by horizontal transmission among adult flies in a density-dependent manner. Further work is needed to deter-

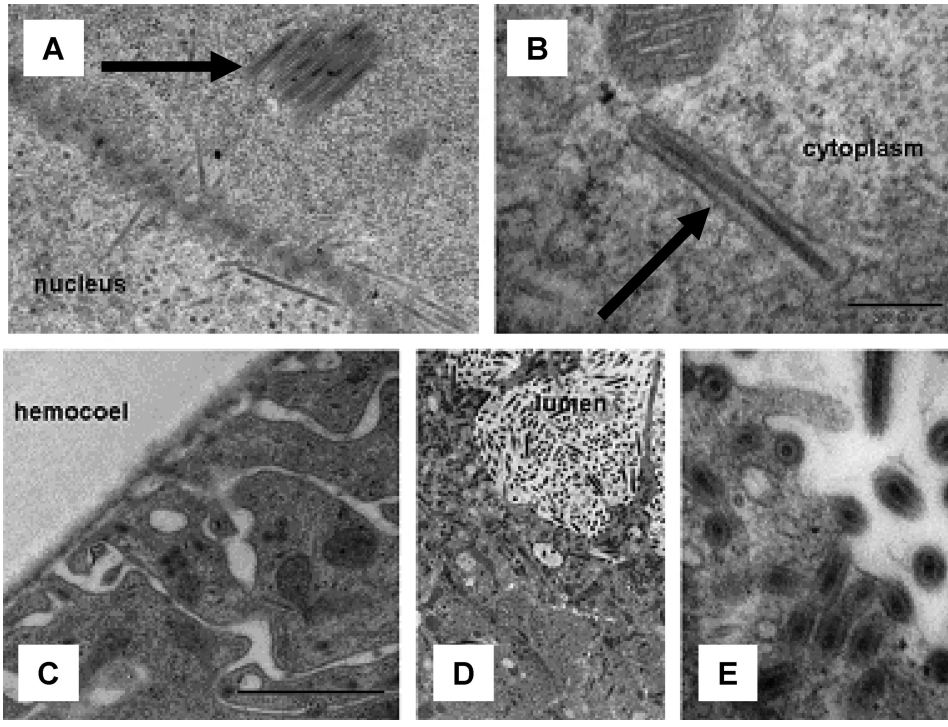

Fig. 3. Transmission electron micrographs of thin sections through an infected salivary gland. Note the production of nucleocapsids in the nucleus and presence of enveloped virus particles in the cytoplasm. Examination of infected cells revealed that the virus displays a directional movement to the luminal surface (note viral budding). Arrows indicate enveloped virus particles. Scale line = 500 nm.

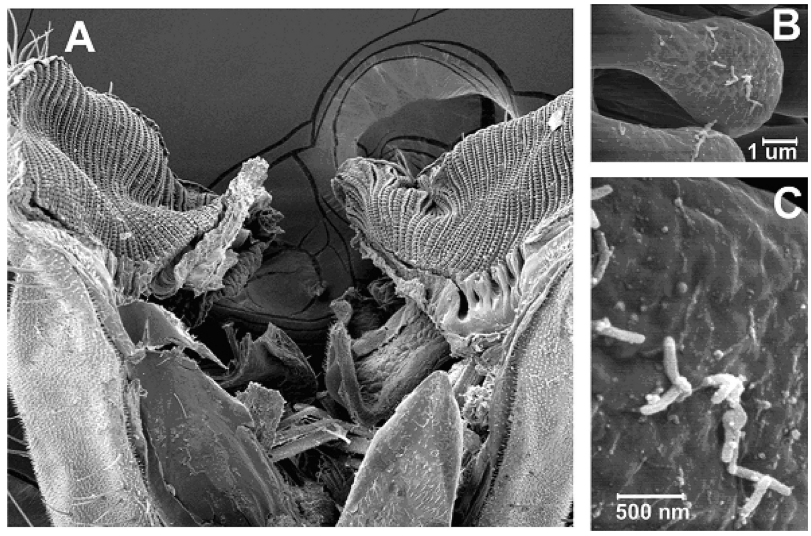

Fig. 4. Scanning electron micrograph of lobes of the labellum of a fly infected with *Md*SGHV (A). High magnification (B, C) revealed the presence of numerous rod shaped particles approximating the size of the enveloped virus.

mine the range of this disease in other geographic areas and in other ecosystems where house flies are common, such as poultry, swine, and landfill operations. If the incidence of infection could be amplified in house fly populations early in the fly season, SGHV could have potential as a population management tool by limiting reproduction of early season population founders. A better understanding of the epizootiology of the virus, especially of conditions conducive to sustaining viability outside the host, is needed to determine its potential in fly management efforts.

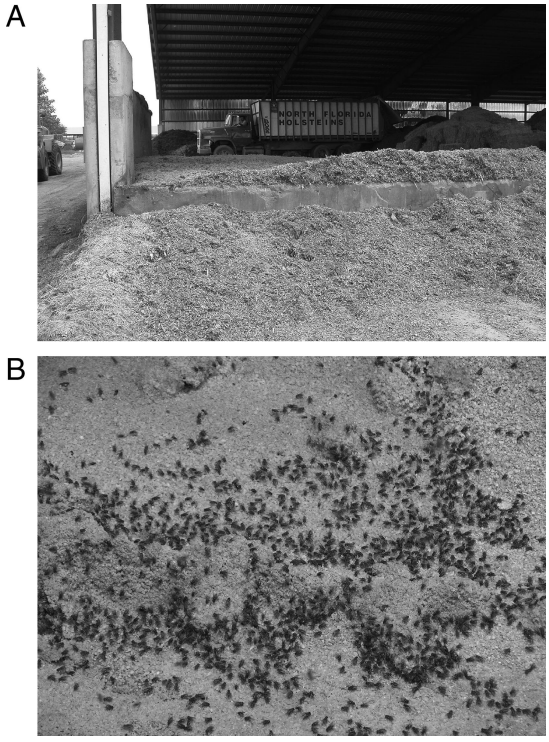

Fig. 5. (A) Brewers grain storage area on farm 1, where incidence of SGHV was highest. (B) Flies feeding on brewers grains.

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